ABSTRACT

Crystalline α-lactose monohydrate and crystalline β-lactose were treated with absolute methanol at room temperature and at reflux temperature. Methanol also was added to fresh aqueous solutions of α- and β-lactose to initiate crystallization. For each of the dried products of these methanol treatments melting point, heat of fusion, and heat capacity (all three by differential scanning calorimetry), density, and crystalline habit were determined. The same measurements were made on the untreated crystalline materials as well as the stable, anhydrous α-lactose obtained by heat treatment. The anhydrous α-lactose α_M, produced by methanol treatment of crystalline α-monohydrate, was different from the stable anhydrous α-lactose α_s formed by heat. The melting point of α_M was lower by 5.8 C, heat of fusion higher by 33%, heat capacity lower by .027 cal g^{-1} deg^{-1}, and density higher by .025 g cm^{-3}. In addition, the various physical measurements offered evidence of several other distinct forms of lactose, a species of β-lactose, β_M, prepared by refluxing in methanol, another β-lactose form β_M', crystallized by methanol, and another anhydrous α-lactose form, α_M', also crystallized by methanol. Each of these three forms had a unique melting point, heat capacity, and density.

INTRODUCTION

The new method from our laboratory for determining crystalline lactose of whey powders by differential scanning calorimetry (DSC) makes use of absolute methanol to pretreat whey powder samples (11). Treatment with methanol for several hours at room temperature with a 20:1 vol/wt ratio of methanol to powder converts α-lactose monohydrate to a stable, anhydrous form (8), which generally is regarded identical to the stable anhydrous form α_s, produced by the heating procedure of Sharp (5, 9, 13).

For calibration, pure crystalline α-lactose monohydrate was dehydrated with methanol according to the analytical protocol. The DSC thermogram of the resultant anhydrous α-lactose indicated a lower melting point and greater heat of fusion than were obtained with α-lactose monohydrate (1). Earlier experiments in our laboratory had shown that α_s had a melting point that was barely distinguishable from α-lactose monohydrate. The unexpected thermograms for methanol-treated lactose suggested that further study of the effects of methanol on lactose be undertaken to determine A) whether the two methods for preparing stable, anhydrous lactose did yield nonidentical products, and B) whether the various forms of lactose differed in other physical properties besides melting point and heat of fusion. Also included in the study was β-lactose, despite its reported insensitivity to anhydrous methanol (9).

MATERIALS AND METHODS

Crystalline α-lactose monohydrate and β-lactose were obtained from Foremost and Eastman Kodak[2], respectively, and were purified according to the method of Buma and van der Veen (4). This purification scheme, involving successive washings with hot glycerol and 95% ethanol, removes nonlactose impurities and adsorbed lactose glass, but in all cases a residual contamination by the other anomes is about 2%. This impurity presumably is incorporated within the crystal structure during growth from solution. The purity of each species was determined by polarimetric analysis (12), which gives both total lactose and the ratio of β to α. For comparison, purity also was determined by

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[1] Agricultural Research Service, U.S. Department of Agriculture.

[2] Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

analysis of the DSC fusion thermograms obtained at a heating rate of 1 C min^{-1} by the procedure of Sondack (14). The DSC and polarimetric analyses for contamination agreed to within .1%; mean values were 2.25% β in the α-lactose monohydrate and 1.95% α in the crystalline β-lactose.

Methanol Treatment

Each of the two purified crystalline lactose samples was subjected to three distinct methanol treatments:

1. Anhydrous methanol was added to the crystals at a ratio of 20:1 (vol/wt), and the mixture was stirred at room temperature for a minimum of 2 to 3 h, as in the analytical procedure for whey powders (11).

2. Anhydrous methanol, again at a 20:1 ratio, was refluxed with the crystalline lactose samples for a minimum of 1 h. A short tube containing Drierite was positioned atop the reflux condenser to exclude moisture from the system.

3. Anhydrous methanol was added to a freshly prepared aqueous lactose solution (10%) so that the final ratio of methanol to solution was 10:1 (vol/vol). For α-lactose, the solution was maintained at room temperature; for β-lactose the temperature was maintained at 90 to 95 C on a hot plate.

The products of each of the three treatments of α- and β-lactose were collected by filtration and dried to constant weight in a vacuum oven at 60 C.

Heat Treatment

In addition to methanol treatment, purified α-lactose monohydrate was dehydrated at 130 C in a closed aluminum weighing dish to give α_s (13).

Calorimetry

A Dupont model 990 Thermal Analyzer in the DSC mode was used to determine melting point (T_m), heat of fusion (ΔH), and heat capacity (Cp) in the range of 20 to 100 C. Instrument calibration procedures were as described previously (11); Cp determinations were further calibrated by highly purified samples of sapphire, aluminum, and benzoic acid.

Fusion thermograms were recorded at 1, 2, 5, 10, and 20 C min^{-1} for the products of the various experimental treatments. A minimum of five replicates at 10 C min^{-1} was used for ΔH determinations. Heats of fusion were calculated from the respective peak areas with a linear baseline across the transition region. Because of the relatively high impurity in each lactose sample (roughly 2% of the other anomer), determination of melting points from the DSC thermograms was ambiguous. Four alternatives may be considered: A) the temperature of first deviation from the low temperature baseline, which is relatively insensitive to heating rate and sample mass; B) the temperature corresponding to the intersection of an extrapolation of the low temperature baseline with an extrapolation of the steepest portion of the fusion peak, a frequently employed choice for T_m characterization which is relatively insensitive to experimental conditions; C) the temperature of maximum differential heating rate, the "peak temperature", which is most variable with heating rate and sample size; and D) the calculated melting point from the Sondack procedure for purity determination (14). At a heating rate of 1 C min^{-1}, this is the intercept on the plot of temperature versus the reciprocal of the fraction melted $(1/F)_T$.

The Sondack method relies on empirical data corrections to give a straight line relationship between T and $(1/F)_T$, and with impurity of 2%, these corrections are necessarily large. The use of option D was rejected because of large empirical data correction terms, even though impurity calculated by this same procedure agreed well with polarimetric analysis for α-lactose monohydrate and crystalline β-lactose. Option A was rejected because of poor precision, and option C was rejected because of its variation with heating rate and sample size. Thus, option B is reported hereafter for each of the lactose samples. Nevertheless, T_m follows the same rank order for the various lactose species regardless of which option is selected. It is the difference between species, and not absolute magnitudes of T_m, which bears emphasis.

Heat capacity was measured at 5 and 10 C min^{-1} in closed aluminum pans with empty pans as reference. A baseline first was obtained with the two pans empty; the DSC cell then was cooled, and a lactose sample of appropriate size (approximately 5 mg) was added to one of the pans. The subsequent Cp run was immedi-

ate without readjustment of instrumental zero point. The Cp at any given temperature is proportional to the difference in pen displacement between sample run and baseline. Heat capacity was calculated for each lactose species at 5 C intervals between 15 and 30 C and at 10 C intervals thereafter to 100 C. Results were fit by the method of least squares to give a linear relationship between Cp and T over 20 to 100 C.

Density

Density was measured by the flotation method of Buma (2) with dispersing mixtures of mineral oil and CCl_4, the densities determined pycnometrically. The volume of sedimented lactose was measured after equilibration at 25.0 C and centrifugation for 5 min in a bench model centrifuge at about 1700 rpm. Sedimentation volume was plotted as a function of density; intersection on the density axis defined lactose density. A reference sample of α-lactose monohydrate was included in each run as a standard. Density measurements by this technique are independent of particle size.

Morphology

Gross morphology was observed microscopically with a polarizing microscope so that birefringence properties also might be observed.

RESULTS

α-Lactose

Figure 1 shows representatuve DSC thermograms of the fusion of α-lactose monohydrate and products of the several methanol treatments, all determined at a heating rate of 10 C min^{-1}. The stable anhydrous α-lactose, prepared by heating in a closed dish, melts with a thermal profile nearly identical to that of the hydrate. Since the water of crystallization within the hydrate vaporizes below 150 C (1), the melting of the hydrate is the melting of the hygroscopic anhydrous form. Even under N_2, pressures of up to 5.28 MPa in a pressure DSC cell, vaporization of hydrate water was a peak between 140 and 150 C. Thus, in Fig. 1 only one thermogram is shown for the α-lactose forms without methanol. Methanol treatments 1 and 2 yielded indistinguishable products, designated hereafter as α_M, presumably the

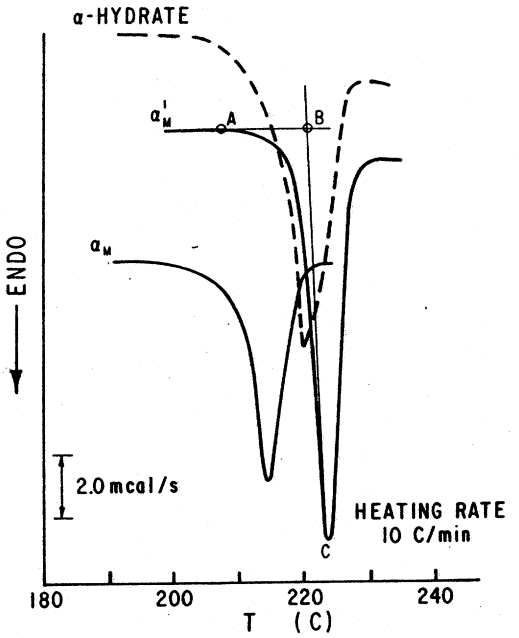

FIG. 1. Representative DSC fusion thermograms of several forms of α-lactose: 6.16 mg α-hydrate, 3.41 mg α_M (product by methanol treatment of α-hydrate), and 4.40 mg α_M' (crystallized from methanol). The points labeled A, B, and C on the thermogram of α_M' refer to alternative conventions for defining the melting point, T_m.

product described by Lim and Nickerson (9). The third thermogram represents the product of treatment 3, designated as α_M', and also indicates the melting temperatures defined by options A, B, and C. As with α_M, the product named α_M' is an anhydrous form of α-lactose as demonstrated by the absence of weight loss in a vacuum oven at 120 C and the absence of a vaporization endotherm by DSC.

Results of other physical measurements are recorded in Table 1, which indicates β-lactose contamination determination by polarimetric and DSC analysis. The ΔH is reported on an anhydrous weight basis. The constants in the linear least squares fit to the Cp data are tabulated along with Cp (25 C) in Table 2.

β-Lactose

Representative DSC fusion thermograms of the several β-lactose species are in Fig. 2. The usual crystalline form is labeled β. Treatment 1 (for as long as 16 h) yielded a product

TABLE 1. Physical properties of α-lactose forms.

Species	Crystalline habit	β-lactose impurity, %	T_M, C[a]	ΔH fusion, cal g^{-1}		Density g cm^{-3}
				Mean	SE	
α-hydrate	Tomahawks[b] + irregular	2.25	216.5	32.2 (34.1)	2.0 (1.7[c])	1.537 (1.535[d], 1.540[e])
α_S	Irregular	2.25	215.8	29.0	.8	1.540 (1.544[e])
α_M'	Irregular	2.40	210.0	39.0	1.1	1.565
α_M	Narrow prisms[b]	.86	220.7	32.9	1.2	1.570 (1.575[e])

[a] Specified by convention B (see text), DSC programming rate of 10 deg min^{-1}.
[b] Birefringent.
[c] Reference 1.
[d] Reference 2.
[e] Reference 14, p. 298.

FIG. 2. Representative DSC fusion thermograms of β-lactose forms: 6.92 mg β, 6.56 mg β_M (produced by refluxing β-lactose in methanol), and 4.73 mg β_M' (crystallized from methanol).

indistinguishable from β in agreement with Lim and Nickerson's results (9), but treatment 2 produced the species labeled β_M. The product of high temperature (90 to 95 C) treatment 3 is β_M'. An impure crop of β_M' may be obtained by methanol crystallization at room temperature. The extent of α impurity in the β_M' closely agrees with that predicted from the mutarotation rate constant (6). Thus, a room temperature solution of initially pure β-lactose will have approximately 7% α after 10 min. Methanol crystallization under such conditions gave 6.5% α impurity in the β_M' crystals.

Results of the other physical measurements are in Table 3. In general, the differences between β and β_M are small but reproducible and greater than the uncertainty in the measurements. For example, the difference in density between β and β_M is extremely small, and the following test was undertaken to verify the difference. A portion of β was dispersed uniformly in an oil and CCl_4 mixture of such

TABLE 2. Heat capacity of various forms of lactose.

Species	Cp (25 C), cal g^{-1} deg^{-1}	A^a		$B^a \times 10^4$	
		Mean	SE	Mean	SE
α-hydrate	.280 (.29[b], .299[c])	.262	.014	7.28	.10
α_s	.291	.280	.108	6.19	.75
α_M	.264	.250	.039	6.23	.34
α_M'	.240	.227	.018	5.63	.17
β	.264 (.285[c])	.248	.013	6.02	.14
β_M	.255	.249	.047	5.43	.25
β_M'	.272	.261	.016	5.32	.20

[a]A and B are constants derived from a least squares fit to the experimental Cp data between 15 and 100 C, such that Cp (T) = A + BT, with T in C.

[b]Reference 3.

[c]Reference 8, page 78.

density that no β would sediment. A small amount of β_M was dropped onto the surface of the liquid and it began to sediment to the bottom of the tube even without centrifugation. Thus, the small difference in density between β and β_M is apparently real.

DISCUSSION

The data for α-lactose indicate little difference between the crystalline monohydrate and α_s, the stable anhydrous form prepared by heat. The differences in melting point, ΔH, and density are real; they are reproducible and ments. Yet they are small and suggest only minor alterations in the structure of the solid. Similarly, the respective Cp values are distinguishable but close; on an anhydrous basis the values of Cp are nearly identical (.295 and .291 at 25 C) and agree with (3, 5).

In contrast, α_M displays strikingly different physical properties. The higher density is indicative of a much more compact, close-fitting structure, which is consistent with the observed higher fusion enthalpy even though melting occurs at a lower temperature.

The properties of α_M' are different still; its density is close to that of α_M, its fusion enthalpy is similar to that of the monohydrate, but its melting point is considerably higher than other forms of α-lactose and Cp much lower. The increase in melting point exceeds the anticipated rise associated with the greater purity of α_M'. That is, if α_M' were merely a more purified form of the monohydrate, or even α_M, then the difference in purity (Table 1) would predict less than 1 C difference in T_m by the thermodynamic expression for melting point depression. Table 1 shows that the actual difference in T_m is greater than 4 C.

The shape of the α_M' crystals is noteworthy since prisms of α-lactose have been mentioned in the literature. Herrington (7) reported that fast crystallization by evaporation produced prisms of α-lactose instead of the characteristic tomahawks. These prisms he assumed to be the monohydrate although he reported no polarimetric analysis. Recently, Majd and Nickerson (10) reported that the initial yield of α-lactose, when supersaturated solutions were diluted with ethanol, was almost entirely in the form of anhydrous α-lactose prisms. Their observations with a polarizing microscope revealed no birefringence. The samples labeled α_M' in this present study showed strong birefringence as intense as that of the monohydrate. However, α_M was neither birefringent nor prismatic. The respective preparation techniques indicate that the anhydrous α-lactose prisms of Majd and Nickerson are probably the same species as α_M', but the discrepancy in birefringence properties is unexplained.

The physical properties of the several forms of β-lactose support the existence of three distinct species, β, β_M, and β_M'. With β-lactose there is little variation in ΔH between species.

TABLE 3. Physical properties of β-lactose forms.

Species	Crystalline habit	α-lactose impurity, %	T_M, C[a]	ΔH fusion, cal g^{-1} Mean	ΔH fusion, cal g^{-1} SE	Density, g cm^{-3}
β	Diamond-shaped plates + irregular[b]	1.95	234.5	50.5 (48.7)	1.5 (1.3[c])	1.584 (1.576[d], 1.59[e])
β_M'	Irregular[b]	1.94	238.0	51.5	1.6	1.588
β_M'	Needles[b]	1.29	230.0	53.0	.8	1.554

[a]Specified by convention B, DSC programming rate of 10 deg min^{-1}.
[b]Birefringent.
[c]Reference 1.
[d]Reference 2.
[e]Reference 14, p. 298.

Density and melting point vary directly whereas Cp varies inversely with density and T_m.

A form of β-lactose needles, crystallized from methanol, was reported by Herrington (7), who described the needles as thin and curved. Majd and Nickerson found no birefringence in their samples of β-lactose needles precipitated by ethanol. The samples of β_M', probably the same species as studied by Majd and Nickerson, were brightly colored under a polarizing microscope. As in the case of α_M', the discrepancy in birefringence observations is unresolved.

SUMMARY

Taken together, the results of physical measurements on the products of methanol treatment of lactose have demonstrated the following:

1. The two methods for producing stable anhydrous α-lactose yield products with dissimilar physical properties. The heat treatment of Sharp produces an anhydrous species which is similar to the monohydrate. Methanol treatment of the monohydrate gives an anhydrous species with distinct density, melting point, ΔH, and Cp.

2. The crystallization of α- or β-lactose by adding methanol to appropriate aqueous lactose solutions results in the rapid formation of anhydrous forms labeled α_M' and β_M' which differ in physical properties from the other forms of lactose. The characteristic crystalline habits of these forms, prisms and needles, have been observed previously, but the other distinct physical properties have not been reported. In contrast to the observations of Majd and Nickerson (10), α_M' and β_M' display strong birefringence.

3. Refluxing in absolute methanol alters the Cp, density, and T_m of β-lactose.

4. The methanol pretreatment of whey powders prior to calorimetric analysis for α-lactose (10) converts all α-lactose to α_M and leaves β-lactose unchanged.

The results of this study further suggest that determination of the actual crystal structure of these various lactose species by x-ray diffraction or infrared spectroscopy might be a fruitful area for future research.

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